at position 44; and a variable light ($V_L$) chain of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and wherein the RFB4 dsFv has 90% or greater of the binding affinity of the prototype RFB4 dsFv.

12. (three times amended) The expression cassette of claim 11, wherein said RFB4 dsFv comprises a $V_H$ of SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a $V_L$ of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

## REMARKS

Claims 1-5, 7-14, 16, 17, 22-27, and 29-32 are pending in the application. With entry of the instant amendment, claims 1, 5, 11, and 12 have been amended. A copy of the currently pending claims is provided in Appendix B, attached hereto.

The amendments to the claims add no new matter and are supported throughout the specification.

Claims 1 and 11 have been amended to recite a variable heavy chain ($V_H$) that has a cysteine at amino acid position 44 and is at least 95% identical to SEQ ID NO:2; and a variable light chain ($V_L$) that has a cysteine at amino acid position 100 and is at least 95% identical to SEQ ID NO:4. Support for the amendment can be found, *e.g.*, at page 18, lines 24-29.

Claims 1 and 11 have been amended to recite a prototype RFB4 dsFv. Support for the amendment can be found, *e.g.*, at page 18, lines 12-29, which describes various Fv fragments, including RFB4dsFv fragments, and further, refers to prototype sequences, SEQ ID NO:2 and SEQ ID NO:4.

For convenience, the rejections will be addressed in the order set out in the Office Action mailed May 8, 2002.

*Objections to claim 1 and the specification*

Claim 1 was objected to because of the typographical error in the term "ds(Fv)". The claim has been amended to recite dsFv, thus obviating the objection.

The specification has been amended to indicate that the application claims benefit of provisional application 60/041,437, filed March 20, 1997.

*Rejection under 35 U.S.C. § 112, first paragraph-enablement*

Claims 1-4, 7, 11, 22-26, and 29-32 stand rejected as allegedly not enabled by the specification. In order to expedite prosecution, the claims have been amended to recited an immunotoxin comprising an RFB4 dsFv that has a $V_H$ with at least 95% identity to SEQ ID NO:2 and a $V_L$ with at least 95% identity to SEQ ID NO:4. Further, the claims recite that the RFB4 dsFv has at least 90% or of the binding affinity of a reference RFB4 dsFv. The rejection maintains that the claims encompass an antibody that does not bind to the same epitope as the RFB4 dsFv, as the antibody only needs to compete for binding which at high enough concentrations such that antibodies with very low affinity for CD22 would bind. To the extent that the rejection applies to the amended claims, Applicants respectfully traverse.

First, Applicants note that the claims include a binding affinity reference standard, that is the RFB4 dsFv must bind with at least 90% of the affinity of a reference RFB4 dsFv. Therefore the Examiner's allegation that antibodies with very low affinity for CD22 are encompassed by the claims is not applicable.

Secondly, the Examiner contends that the antibody of claims 1 and 11, could bind to a different epitope and mask the epitope bound by the RFB4 antibody and thus, would not bind to the same epitope. The claims are drawn to variable regions have at least 95% identity to reference light chain and heavy chain variable sequences. The reference sequence provides a structural characteristic of the feature of the claimed RFB4 dsFv antibodies. Further, a functional characteristic of the claimed sequences (at least 90% binding affinity) is also provided. The specification provides guidance to determine the percent identity (*see, e.g.,* page 13, line 1 through page 14, line12) and guidance in

performing binding assays (*see, e.g.,* page 21, lines 6-23, ), both of which techniques are well known in the art. One of skill in the art could thus determine antibody fragments that can be used in constructing the currently claimed immunoconjugates. The Examiner has presented no evidence or reasoning as to why the practitioner could not identify an RFB4 dsFv sequence that shares the structural and functional characteristics of the RFB4 dsFvs set forth in the claims. Therefore, although, the amount of experimentation required by one of skill to perform these assays may be time consuming, it is not undue in view of the guidance provided in the specification.

Lastly, the Examiner alleges that Applicants' response of March 7, 2002 does not address Greenspan *et al*, which was cited in a previous Office Action as allegedly teaching that it is difficult to map an epitope. The Examiner then concluded that one of skill in the art could not reasonably be expected to be able to map an epitope at the time of the invention. Applicants note that Greenspan was in fact addressed on page 6 of Applicants' response filed March 7, 2002. In addition, Applicants submit that Greenspan merely points out that binding of an antibody to an epitope is complex. The rejection provides no evidence as to why Greenspan would suggest or teach that one of skill could not determine, one by one, RFB4 dsFv variants that have 95% identity to the references sequences and the claimed binding properties.

Thus, in view of the structural and functional characteristics of the claimed sequences, the guidance provided in the specification, and the level of skill in the art, the claims are enabled. Applicants therefore respectfully request withdrawal of the rejection.

*Rejections under 35 U.S.C. § 112, first paragraph-written description*

Claims 1-5, 7-14, 16-17, 22-27, and 29-32 were rejected as allegedly lacking adequate written description. To the extent that the rejection applies to the amended claims, Applicants respectfully traverse. The rejection alleges that there is an element set out in the claims that recites an immunoconjugate that has 90% or greater of the binding affinity of the RFB4 dsFv and that this is not found at page 15, lines 10-14. Applicants note that claims 1 and 11 are drawn to immunoconjugates comprising an

RFB4 dsFv with the claimed binding specificity. This is supported in the indicated passage. Applicants therefore respectfully request withdrawal of the rejection.

*Rejections under35 U.S.C. §103*

Claims 1-5, 7-14, 16, 17, 22-27, and 29-32 stand rejected as allegedly unpatentable over the various references cited by the Examiner on page 6 of the Office Action. The rejection contends that it would have been *prima facie* obvious to obtain the DNA and protein sequences of the $V_H$ and the $V_L$ from the RFB4 hybridoma using the method of Orlandi *et al*, and then use the method of inhibiting B-cells as taught by Ghetie *et al.* in conjunction with the methods of Reiter *et al.* and Kuan *et al.* to produce a disulfide stabilized anti-CD22 immunoconjugate.

Although the Examiner acknowledges that the references do not teach the amino acid sequences of SEQ ID NOs:2 and 4, he alleges that the sequences would have been obvious over the hybridoma taught by Shen in view of the methodology taught by Orlandi *et al.* Applicants respectfully traverse.

As noted in Applicants' previous responses, *e.g.*, pages 4 and 5 of the response filed March 7, 2007, the Examiner's position that the sequences are obvious over the disclosure of the hybridoma and a technique to PCR variable regions is inconsistent with the holdings of the Federal Circuit. Further, even assuming *arguendo* the sequences were obvious, the art does not predict an RFB4 immunotoxin that has the expression and cytotoxicity properties of the claimed sequences.

*The amino acid sequences are unobvious*

The rejection alleges that Orlandi *et al.* teaches a general technique for obtaining the $V_H$ and the $V_L$ genes, which in conjunction with the teachings of Shen *et al.* describing the hybridoma, render SEQ ID NOs:2 and 4 obvious.

As noted in Applicants response of March 7, 2002, the Federal Circuit has held that a nucleic acid sequence is not obvious over general methods of isolating cDNA or DNA molecules (*see, e.g., In re Deuel*, 34 USPQ2d, 1210 (Fed Circ. 1995)). The

Examiner appears to argue that the Federal Circuit rulings are only applicable for a "general nucleic acid sequence" (page 10 of the Office Action) and do not apply to the instant claims because of homology in $V_H$ and $V_L$ genes, which permits design of primers that could be allegedly used for PCR to obtain the sequences. Applicants submit that the Examiner's understanding of *Deuel* is incorrect. The issue in *Deuel* was whether knowledge of the partial amino acid sequence of a protein, in conjunction with a reference indicating a general method of cloning, rendered the sequences obvious. The Federal Circuit reversed a decision by the Board of Patent Appeals that the sequences were obvious. In reversing the decision, the Court explained that

> Because [appellant] claims new chemical entities in structural terms, a *prima facie* case of unpatentability requires that the teachings of the prior art suggest the claimed compounds to a person of ordinary skill in the art. Normally a *prima facie* case of obviousness is based upon structural similarity, *i.e.*, an established structural relationship between a prior art compounds and the claimed compound. Structural relationship may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties...In all these cases, however, the prior art teaches a specific, structurally definable compound and the question becomes whether the prior art would have suggested making the specific molecular modifications necessary to achieve the claimed invention.

(*In re Deuel*, at 1214, emphasis added). The Court further stated that "no particular one of these DNA can be obvious unless there is something in the prior art to lead to the particular DNA" (emphasis added).

Orlandi *et al.* teach only a PCR method of obtaining some fragments encoding $V_H$ and $V_{kappa}$ genes using primers that were developed based on consensus sequences that they identified. They do not teach that any particular sequences would be obtained, nor do they teach that all $V_H$ and $V_L$ sequences could be obtained using their methodology. Moreover, Orlandi *et al.* specifically point out that, using their method, "it is not possible to determine the exact sequence of both ends of the V genes" (page 2837,

column 1, sentence 1, emphasis added). Therefore, using the methods of Orlandi *et al.*, one of skill could not expect to obtain the sequences SEQ ID NO:2 and SEQ ID NO:4. Thus, Orlandi *et al.* do not provide teachings that render the claimed invention obvious.

*The prior art does not predict an immunotoxin with the superior expression and cytotoxicity properties of the claimed immunotoxin.*

The prior art also does not teach or predict a recombinant immunotoxin having the particular properties of the claimed recombinant immunotoxins, *e.g.,* the stability and cytotoxicity. As explained in Applicants response of March 7, 2002, the art provides evidence that there is variation in the stability and cytotoxicity of immunotoxin comprising various antibodies (*see, e.g.,* page 5 and 6 of the response).

The Examiner aregues that Reiter *et al.* (*Nature Biotech*) is directed to comparison of dsFv-Pe38 conjguates to IgG, scfv-PE38 and Fab fragment. He alleges that the reference does not compare the dsFv to the dsFv-Pe38 immunoconjugate. However, Applicants note that the claims recite an RFB4dsFv having at least 90% of the affinity of the reference RFB4. Thus, that aspect of the rejection does not apply to the claims.

The Examiner also argues that Reiter *et al.* (*Biochemistry* 33:5451-59, 1994) teach dsFv-immunotoxins that have equal or improved and activity and that 4 out of 8 dsFV-immunotoxins described in Reiter *et al.* (*Nature Biotech*) have improved binding affinity. He concludes that one of skill would therefore expect these properties. Applicants respectfully disagree. While the art may indicate that some dsFv immunotoxins may have superior stability and cytotoxicity, it does not predict which antibody can be stably expressed as immunoconjugates to provide superior stability and cytotoxicity. Indeed, there is tremendous variability in the stability and cytotoxicity of these immunoconjugate constructs, as evidenced by the data presented in Table 3 of Reiter *et al.* (*Nature Biotech.*). No evidence is provided in the rejection that the practitioner can predict which antibody will work well. Once such an antibody has been identified, it is then a matter of routine experimentation to identify variants of that

antibody that have the same properties, as explained in the section above addressing the enablement rejection.

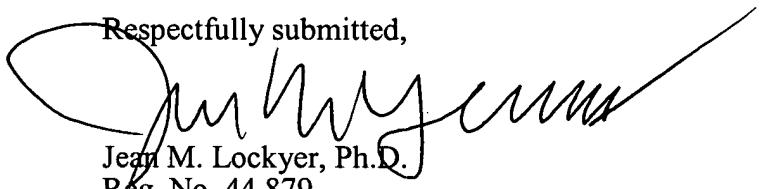
For the reasons set forth above, the claimed RFB4dsFV immunotoxins are unobvious. Applicants respectfully request withdrawal of the rejection.

## CONCLUSION

Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at .

Respectfully submitted,



Jean M. Lockyer, Ph.D.
Reg. No. 44,879

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: (415) 576-0300
JML:jml
SF 1389989 v1

## APPENDIX A

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (three times amended) A recombinant immunoconjugate, comprising a therapeutic agent or a detectable label covalently linked to a recombinant RFB4 disulfide-stabilized Fv (dsFv) [antibody that binds an extracellular epitope of CD22 (an "anti-CD22 antibody")] having a variable heavy chain ($V_H$) with a cysteine at amino acid position 44, which heavy chain is at least 95% identical to SEQ ID NO:2; and a variable light chain ($V_L$)with a cysteine at amino acid position 100, which light chain is at least 95% identical to SEQ ID NO:4; wherein [the anti-CD22 antibody is a] the RFB4 dsFv [binding fragment that] competes for binding to CD22 [to a same epitope as an] with a prototype RFB4 dsFv [disulfide-stabilized Fv (dsFv)] comprising a variable heavy ($V_H$) chain [as set out in] of SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light ($V_L$) chain [as set out in] of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and wherein the RFB4 dsFv has 90% or greater of the binding affinity of the prototype RFB4 dsFv [ds(Fv)].

5. (three times amended) The recombinant immunoconjugate of claim 1, wherein said RFB4 dsFv [anti-CD22 antibody is an RFB4 disulfide-stabilized Fv (dsFv) comprising a] comprises a $V_H$ of SEQ ID NO:2 [variable heavy ($V_H$) chain as set out in SEQ ID NO:2], in which a Cys residue is substituted for Arg at position 44; and a $V_L$ of SEQ ID NO:4 [variable light ($V_L$) chain as set out in SEQ ID NO:4], in which a Cys residue is substituted for Gly at position 100.

11. (three times amended) An expression cassette encoding a recombinant immunoconjugate comprising a sequence encoding for a toxin peptide and an antibody that binds to an RFB4 disulfide-stabilized Fv (dsFv) having a variable heavy chain ($V_H$) with a cysteine at amino acid position 44, which heavy chain is at least 95% identical to SEQ ID NO:2; and a variable light chain ($V_L$) with a cysteine at amino acid

position 100, which light chain is at least 95% identical to SEQ ID NO:4; wherein the RFB4 dsFv competes for binding to CD22 with a prototype RFB4 dsFv comprising a variable heavy ($V_H$) chain of SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light ($V_L$) chain of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and wherein the RFB4 dsFv has 90% or greater of the binding affinity of the prototype RFB4 dsFv. [extracellular epitope of CD22 (an "anti-CD22" antibody) having a $V_H$ encoding for a cysteine at amino acid position 44 and a $V_L$ encoding for a cysteine at amino acid position 100; wherein the anti-CD22 antibody is a binding fragment that competes for binding to a same epitope as an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy ($V_H$) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light ($V_L$) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and has 90% or greater of the binding affinity of the RFB4 ds(Fv).]

12. (three times amended) The expression cassette of claim 11, wherein said RFB4 dsFv [antibody is an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy ($V_H$) chain as set out in] comprises a $V_H$ of SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a [variable light ($V_L$) chain as set out in] $V_L$ of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

## APPENDIX B

## CURRENTLY PENDING CLAIMS

1. (three times amended) A recombinant immunoconjugate, comprising a therapeutic agent or a detectable label covalently linked to a recombinant RFB4 disulfide-stabilized Fv (dsFv) having a variable heavy chain ($V_H$) with a cysteine at amino acid position 44, which heavy chain is at least 95% identical to SEQ ID NO:2; and a variable light chain ($V_L$)with a cysteine at amino acid position 100, which light chain is at least 95% identical to SEQ ID NO:4; wherein the RFB4 dsFv competes for binding to CD22 with a prototype RFB4 dsFv comprising a variable heavy ($V_H$) chain of SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light ($V_L$) chain of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and wherein the RFB4 dsFv has 90% or greater of the binding affinity of the prototype RFB4 dsFv.

2. (as filed) The recombinant immunoconjugate of claim 1, wherein said therapeutic agent is a toxin.

3. (as filed) The recombinant immunoconjugate of claim 2, wherein said toxin is a *Pseudomonas* exotoxin (PE) or a cytotoxic fragment thereof.

4. (as filed) The recombinant immunoconjugate of claim 3, wherein said cytotoxic fragment is PE38.

5. (three times amended) The recombinant immunoconjugate of claim 1, wherein said RFB4 dsFv comprises a $V_H$ of SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a $V_L$ of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

7. (once amended) The recombinant immunoconjugate of claim 3, wherein said variable heavy ($V_H$) chain is covalently linked to the carboxyl terminus of said toxin.

8. (twice amended) The recombinant immunoconjugate of claim 5, wherein said $V_H$ chain is covalently linked to said $V_L$ chain through a linker peptide.

9. (once amended) The recombinant immunoconjugate of claim 5, wherein said $V_H$ chain is linked to said $V_L$ chain through a cysteine-cysteine disulfide bond.

10. (as filed) The recombinant immunoconjugate of claim 8, wherein said linker peptide has the sequence of SEQ ID NO:5.

11. (three times amended) An expression cassette encoding a recombinant immunoconjugate comprising a sequence encoding for a toxin peptide and an antibody that binds to an RFB4 disulfide-stabilized Fv (dsFv) having a variable heavy chain ($V_H$) with a cysteine at amino acid position 44, which heavy chain is at least 95% identical to SEQ ID NO:2; and a variable light chain ($V_L$) with a cysteine at amino acid position 100, which light chain is at least 95% identical to SEQ ID NO:4; wherein the RFB4 dsFv competes for binding to CD22 with a prototype RFB4 dsFv comprising a variable heavy ($V_H$) chain of SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light ($V_L$) chain of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and wherein the RFB4 dsFv has 90% or greater of the binding affinity of the prototype RFB4 dsFv.

12. (three times amended) The expression cassette of claim 11, wherein said RFB4 dsFv comprises a $V_H$ of SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a $V_L$ of SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

13. (as filed) The expression cassette of claim 11, wherein said toxin is a *Pseudomonas* exotoxin (PE) or a cytotoxic fragment thereof.

14. (as filed) The expression cassette of claim 11, wherein said cytotoxic fragment is PE38.

16. (once amended) The expression cassette of claim 12, further comprising a sequence encoding for a linker peptide having the sequence of SEQ ID NO:5.

17. (as filed) A host cell comprising an expression cassette of claim 11.

22. (once amended) A method for inhibiting the growth of a malignant B-cell that expresses a CD22 molecule on the surface of the cell, said method comprising:

contacting said malignant B-cell with an effective amount of a recombinant immunoconjugate of claim 1, thereby inhibiting the growth of the malignant B-cell.

23. (as filed) The method of claim 22, wherein said toxin is a *Pseudomonas* exotoxin (PE) or a cytotoxic fragment thereof.

24. (as filed) The method of claim 22, wherein said malignant B-cell is contacted *in vivo*.

25. (as filed) The method of claim 22, wherein said malignant B-cell is selected from the group consisting of: a rodent B-cell, a canine B-cell, and a primate B-cell.

26. (as filed) The method of claim 23, wherein said cytotoxic fragment is a PE38 fragment.

27. (twice amended) The method of claim 22, wherein said immunoconjugate comprises an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy ($V_H$) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light ($V_L$) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

29. (amended) The method of claim 23, wherein a variable heavy chain is covalently linked at the carboxyl terminus of said toxin.

30. (amended) The method of claim 29, wherein said $V_H$ chain is covalently linked to said $V_L$ chain through a linker peptide.

31. (as filed) The method of claim 29, wherein said $V_H$ chain is linked to said $V_L$ chain through a cysteine-cysteine disulfide bond.

32. (as filed) The method of claim 31, wherein said linker peptide has the sequence of SEQ ID NO:5.